

Purification and biochemical characterization of human pluripotent hematopoietic colony-stimulating factor

(protein purification/hematopoietic stem cells)

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Communicated by Lewis Thomas, October 24, 1984

ABSTRACT Pluripotent hematopoietic colony-stimulating factor (pluripotent CSF), a protein that is constitutively produced by the human bladder carcinoma cell line 5637, has been purified from low serum (0.2% fetal calf serum)-containing conditioned medium. The purification involved sequential ammonium sulfate precipitation, ion-exchange chromatography, gel filtration, and reversed-phase high-performance liquid chromatography. The purified protein has a molecular weight of 18,000 in NaDodSO₄/polyacrylamide gel electrophoresis, both by the silver staining technique and by elution of biological activity from a corresponding gel slice, and has an isoelectric point of 5.5. Pluripotent CSF supports the growth of human mixed colonies, granulocyte-macrophage colonies, and early erythroid colonies and induces differentiation of the human promyelocytic leukemic cell line HL-60 and the murine myelomonocytic leukemic cell line WEHI-3B (D+). The specific activity of the purified pluripotent CSF in the granulocyte-macrophage colony assay is 1.5×10^8 units/mg of protein.

Colony-stimulating factors (CSFs) are hormone-like glycoproteins produced by a variety of tissues and tumor cell lines that regulate hematopoiesis and are required for the clonal growth and maturation of normal bone marrow cell precursors *in vitro* (1, 2). In contrast to the murine system (3-6), human CSFs have been less well characterized, both biologically and biochemically (7-12). Purification to apparent homogeneity has only been reported for macrophage active CSF (CSF-1) (13, 14), erythroid-potentiating-activity (30), and possibly for granulocyte-macrophage CSF (GM-CSF) (15), but not for human pluripotent CSF.

Assays are available to detect human clonogenic precursors that give rise to cells of the erythroid, granulocytic, megakaryocytic, macrophage (CFU-GEMM) (16, 17), and possibly lymphoid (18) lineages. CSFs with activities on these multipotential progenitor cells (pluripotent CSF) are produced by mitogen- or antigen-activated T lymphocytes (19) and by human tumor cell lines: SK-Hep (unpublished data); 5637 bladder carcinoma cell line (reported in this paper); and human T-cell leukemia virus-transformed lymphoid cells (20, 21, 30). Pluripotent CSF is involved in the proliferation and differentiation of pluripotent progenitor cells leading to the production of all major blood cell types.

We report in this paper the purification and biochemical characterization of a human pluripotent CSF, produced and released by the human bladder carcinoma cell line 5637.

MATERIALS AND METHODS

Assay for GM-CSF Activity. GM-CSF activity was tested on human bone marrow cells (BM cells) cultured with serial dilutions of test samples in semi-solid agar. BM from healthy

human volunteers, who gave informed consent, was diluted 1:5 in phosphate-buffered saline (P_i/NaCl; 20 mM phosphate/0.15 M NaCl) and separated by density gradient centrifugation on Ficoll-Hypaque. Separated cells (10^5) were plated in 1 ml of 0.3% agar culture medium that included supplemented McCoy's 5A medium and 10% heat-inactivated fetal calf serum (FCS), as described (22). To this mixture serial dilutions of a laboratory standard or test samples (10%; vol/vol) in RPMI 1640 medium with 10% FCS were added. Cultures were scored for colonies (>40 cells per aggregate) and morphology was assessed after 7 and 14 days of incubation. GM-CSF units were determined from dose-response curves and expressed as units (u)/ml, where 50 u is the CSF concentration stimulating half-maximal colony number to develop (3).

Assay for CSF for Early Erythroid Colonies (BFU-E) and CFU-GEMM. The colony assay for human BFU-E and CFU-GEMM was performed as described (23). Human BM cells were subjected to a density cut with Ficoll-Hypaque (density, 1.077 g/cm³; Pharmacia) and the low density cells were suspended in RPMI 1640 medium containing 10% FCS at 2×10^7 cells per ml and placed for adherence on Falcon tissue culture dishes (no. 3003, Becton Dickinson, Cockeysville, MD) for 1½ hr at 37°C. The nonadherent cells were depleted of T lymphocytes by rosetting with neuraminidase-treated sheep erythrocytes. Medium conditioned by leukocytes from patients with hemochromatosis in the presence of 1% (vol/vol) phytohemagglutinin (23) as positive control or serial dilutions of test samples were then added at 5% (vol/vol) to 5×10^4 of these low density, nonadherent, and T-lymphocyte-depleted BM cells in a 1-ml mixture of Iscove's modified Dulbecco medium (GIBCO), 0.8% methylcellulose, 30% FCS, 0.05 mM 2-mercaptoethanol, 0.2 mM hemin, and 1 u of erythropoietin (Hyclone, Logan, UT, or Connaught Laboratories, Willowdale, ON). The addition of hemin is necessary to obtain optimal cloning efficiency (24). Dishes were incubated in a humidified atmosphere of 5% CO₂ in air at 37°C. After 14 days of incubation, colonies were scored and morphology was assessed.

As shown in *Results*, a single protein stimulates colony formation by CFU-GEMM, BFU-E, and granulocyte-macrophage colony (CFU-GM) progenitor cells. This protein we termed "pluripotent CSF." Due to the low numbers of mixed colonies per dish attainable in this assay system, titration of test samples for determination of pluripotent CSF activity meets with considerable difficulties. Therefore, we used the GM-CSF assay as described above to measure the GM-CSF aspect of the pluripotent CSF activity in those samples that supported growth of BFU-E and CFU-GEMM for calculat-

Abbreviations: CFU-GEMM, colony-forming unit—granulocyte, erythroid, macrophage, megakaryocyte; CFU-GM, colony-forming unit—granulocyte-macrophage; BFU-E, erythroid burst-forming unit; GM-CSF, granulocyte-macrophage colony-stimulating factor; u, unit(s); BM cells, bone marrow cells; RP-HPLC, reversed-phase HPLC; FCS, fetal calf serum; IEF, isoelectrofocusing.

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ing the specific activity through the purification procedure.

Differentiation Induction Assay. Titrated samples of purified pluripotent CSF were assayed for differentiation induction of WEHI-3B (D+) or HL-60 leukemic cells as described (25).

Preparation of 5637 Cell Line Conditioned Medium (5637 C Medium). The human bladder carcinoma cell line 5637 has been reported to produce a CSF for granulocytes and macrophages (26). The cell line has been maintained in this institute for several years. It is serially passaged by trypsinization in the presence of EDTA and grows rapidly to form an adherent monolayer in plastic tissue culture flasks. Routinely, cells are cultured in RPMI 1640 medium, supplemented with 2 mM L-glutamine, antibiotics, and 10% FCS. For purification of pluripotent CSF activity from 5637 C medium, confluent cell cultures were intermittently cultured in medium containing 0.2% FCS. After 48–72 hr, 5637 C medium was harvested, and cells and cell debris were removed by centrifugation (20 min, $10,000 \times g$) and stored at -20°C until use.

Ammonium Sulfate Precipitation, Ion-Exchange Chromatography, and Gel Filtration. The first three purification steps [$(\text{NH}_4)_2\text{SO}_4$ precipitation, ion-exchange chromatography on DE-52 DEAE-cellulose (Whatman), and gel filtration on Aca 54 Ultrogel (LKB)] were performed as described in detail for interleukin 2 (27) with the exception that Aca 54 was used instead of Aca 44 (see also legends to Figs. 1 and 2).

Reversed-Phase HPLC (RP-HPLC). RP-HPLC was performed with a Waters HPLC system (M 6000 solvent delivery pumps, model 400 variable wavelength detector, data module, and data processor, Waters Associates). The separation was performed on a μ Bondapak C_{18} column (Waters Associates). The buffers used were buffer A (0.9 M acetic acid/0.2 M pyridine, pH 4.0) and buffer B [buffer A in 50% 1-propanol (Burdick and Jackson, Muskegon, MI)]. Acetic acid and pyridine were purchased from Fisher. The pluripotent CSF-containing pool obtained from gel filtration was acidified with acetic acid to pH 4.0 and injected onto the μ Bondapak C_{18} column without regard to sample volume. The column was washed with buffer A (20 min) and bound proteins were eluted by using a steep gradient of 0–40% buffer B within the first 20 min and a 40–100% gradient of buffer B in 120 min. The percentage of buffer A was inversely proportional to buffer B. The flow rate was adjusted to 1 ml/min and 3-ml fractions were collected. From each fraction a 0.5-ml aliquot was supplemented with 10% FCS, dialyzed against P_i/NaCl , and tested for pluripotent CSF activity.

Isoelectrofocusing (IEF). One milliliter of the purified pluripotent CSF was supplemented with 20% glycerol (vol/vol) and 2% Ampholine (vol/vol) at pH 3.5–10 (LKB). A 5–60% glycerol density gradient containing 2% Ampholine (pH 3.5–10) was layered into an IEF column (LKB 8100). The pluripotent CSF sample was applied onto the isodense region of the gradient, followed by IEF (2000 V, 24 hr). Five-milliliter fractions were collected and the pH was determined in each fraction. The fractions were dialyzed against P_i/NaCl and subsequently tested for pluripotent CSF activity.

NaDodSO₄/Polyacrylamide Gel Electrophoresis (NaDodSO₄/PAGE). The discontinuous Tris/glycine system of Laemmli (28) was used for 1.5-mm slab gels of 15% acrylamide. The samples (200 ng of lyophilized protein eluted from HPLC) were treated with 1% NaDodSO₄ in 0.0625 M Tris-HCl (pH 6.8) at 37°C for 1 hr under both reducing (5% 2-mercaptoethanol) and nonreducing conditions and then loaded on the gel. After electrophoresis, gels were stained by the Bio-Rad silver staining method (Bio-Rad). Apparent molecular weights were determined by using protein standards: ovalbumin (M_r 43,000), chymotrypsinogen (M_r 25,700), β -lactoglobulin (M_r 18,400), lysozyme (M_r 14,300), and cyto-

chrome *c* (M_r 12,300) (Bethesda Research Laboratories). After treatment (see above) of lyophilized pluripotent CSF under nonreduced conditions and subsequent electrophoresis, parallel gels were sliced in 4-mm or 2-mm sections, respectively, and proteins from each slice were eluted either into 0.5 ml of RPMI 1640 medium containing 10% FCS or into P_i/NaCl . After extensive dialysis, the eluted material was assayed for pluripotent CSF activity.

Protein Assay. The protein content of samples was measured by using the Lowry technique (29). For protein concentrations of $<2 \mu\text{g/ml}$, samples were subjected to NaDodSO₄/PAGE, the protein bands were visualized by the silver staining technique, and the protein concentration was estimated by comparison with a serial dilution of known amounts of proteins.

RESULTS

Pluripotent CSF Activity in 5637 C Medium. Confluent layers of 5637 human bladder carcinoma cells, when cultured for 48–72 hr in the presence of 10% FCS, released into the culture medium 3000–10,000 u of GM-CSF activity per ml. Media conditioned in the presence of 0.2% FCS still contained 10–30% of this activity, whereas in serum-free 5637 C medium the activity fell below 5% of the activity obtained in the presence of 10% FCS (data not shown). Although GM-CSF activity in 5637 C medium is readily detectable in soft agar BM cultures, not all batches of unfractionated 5637 C medium support *in vitro* growth of BFU-E and CFU-GEMM. Four to $10\times$ concentrated 5637 C medium reduced colony formation by CFU-GM 30–70%, indicating the presence of inhibitor(s) in 5637 C medium. Inhibitors were removed after ion-exchange chromatography.

Purification of Pluripotent CSF. A 20-fold concentration of proteins from the 5637 C medium was achieved by precipitation with $(\text{NH}_4)_2\text{SO}_4$ at 80% saturation. The dialyzed precipitate was loaded on to a DEAE-cellulose (DE-52) column. Bound proteins were eluted with a salt gradient from 0.05 to 0.3 M NaCl in 0.05 M Tris-HCl (pH 7.8). GM-CSF activity eluted as peak 1 between 0.075 M and 0.1 M NaCl and with a second peak at 0.13 M NaCl (Fig. 1). Since only peak 1 revealed pluripotent CSF activity, we used only this pool for

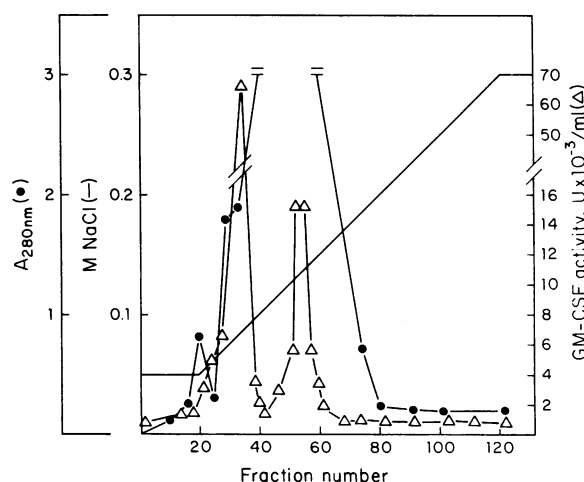


Fig. 1. Ion-exchange chromatography. One liter of dialyzed $(\text{NH}_4)_2\text{SO}_4$ precipitate of 5637 C medium was applied in 0.05 M Tris-HCl (pH 7.8) on a 1-liter DEAE-cellulose (DE-52) column. Bound proteins were eluted with a linear gradient of NaCl (0.05–0.3 M) in 0.05 M Tris-HCl (pH 7.8) as indicated (—). The elution of proteins was monitored by absorption at 280 nm (●) and each fraction was tested for CSF activities (GM-CSF activity; Δ). Proteins from the first peak of GM-CSF activity eluted from the column gave rise to mixed colonies in a CFU-GEMM assay and were used for further purification (pluripotent CSF).

Table 1. Purification of human pluripotent CSF

Fraction	Protein	Total activity*, u $\times 10^{-6}$	Specific activity, u/mg	Purification, fold	Yield, %
5637 C medium	2 g	12	6.0×10^3	—	100
DEAE-cellulose	300 mg	5	1.7×10^4	1 [†]	42
Aca 54 Ultrogel	13 mg	3.1	2.4×10^5	14	26
RP-HPLC	5 μ g	0.74	1.5×10^8	9000	6.2

*GM-CSF activity of pluripotent CSF.

[†]Estimate of fold purification based on starting activity of peak 1 of DEAE-cellulose chromatography.

further purifications. Peak 2 included proteins with only GM-CSF activity. We calculated the "fold" purification by measuring the GM-CSF activity of pluripotent CSF. In the unfractionated 5637 C medium we could not discriminate between GM-CSF activity as part of pluripotent CSF activity and GM-CSF activity without pluripotent properties. Therefore, we considered the GM-CSF activity contained in peak 1 from DE-52 as the starting activity (Table 1).

Since in the subsequent purification schedule GM-CSF, BFU-E, and CFU-GEMM activities copurified in all steps, we named these combined activities pluripotent CSF and have used this term thereafter. The proteins of peak 1 of DE 52 chromatography (including pluripotent CSF activity) were concentrated by dialyzing against 50% (wt/vol) polyethyleneglycol in P_i /NaCl and purified further by Aca 54 Ultrogel gel filtration. The pluripotent CSF activity eluted in fractions 42–49 as a single peak corresponding to a M_r of 32,000 (Fig. 2). This step resulted in a 65% recovery of activities and a 15-fold increase of specific activities (Table 1). The final step involved chromatography on a RP-HPLC column (μ Bondapak C_{18}). The majority of proteins did not bind to this column (not shown) or eluted at low 1-propanol concentrations (<20% 1-propanol; Fig. 3). A minor peak of GM-CSF activity without activity in the CFU-GEMM and BFU-E assays but differentiation inducing activity on HL-60 leukemic cells (unpublished observation) was eluted at around

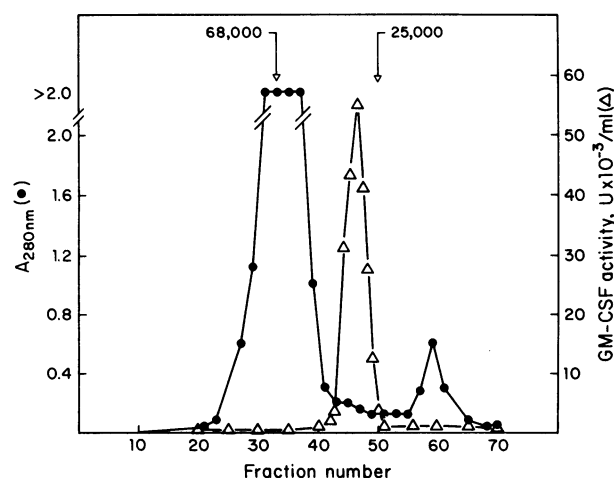


FIG. 2. Gel filtration chromatography. The pluripotent CSF-containing concentrated pool of DEAE-cellulose chromatography was loaded on an Aca 54 Ultrogel column (2.6 \times 90 cm) and eluted with P_i /NaCl. Arrows denote the elution points of bovine serum albumin (M_r 68,000) and chymotrypsinogen (M_r 25,000). The elution of proteins was monitored by absorption at 280 nm (●) and each fraction was tested for pluripotent CSF activity (GM-CSF activity: Δ).

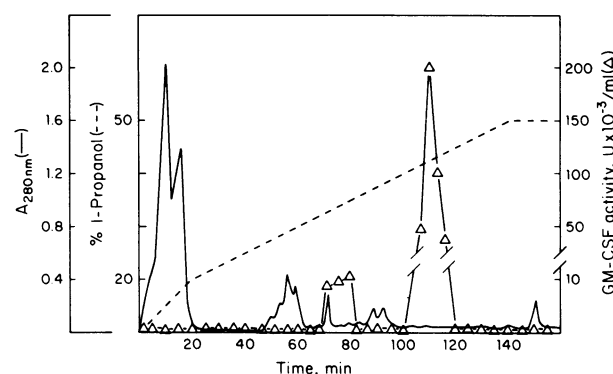


FIG. 3. RP-HPLC. The pooled fractions with pluripotent CSF activities eluted from the gel filtration column were acidified to pH 4.0 and loaded onto a C_{18} (μ Bondapak, Waters Associates) column. The bound proteins were eluted with a linear gradient of 1-propanol in 0.9 M acetic acid/0.2 M pyridine, pH 4.0. The elution of proteins was monitored by absorption at 280 nm (—) and each fraction was tested for pluripotent CSF activity (GM-CSF activity: Δ).

30% 1-propanol. Pluripotent CSF activity eluted as a single sharp peak at 42% 1-propanol (Fig. 3). This purification step resulted in a 600-fold increase of specific activity and a 25% recovery of activity. The protein content of the HPLC fraction was measured by comparing the density in silver-stained NaDodSO₄/PAGE gels with protein standards of known concentrations. Using this measurement, we obtained a specific activity of 1.5×10^8 u/mg of protein and a final purification of 9000-fold, calculated from the first peak of DEAE-cellulose chromatography. The overall yield was 6.2%. Purification with the degree of purification of pluripotent CSF as measured by GM-CSF activity, protein content, specific activity, and yield is detailed in Table 1.

The final preparation obtained after HPLC (pluripotent CSF activity peak fraction) was analyzed on a 15% NaDodSO₄/PAGE gel followed by the sensitive silver staining technique (Fig. 4). Only one major protein band with a M_r of 18,000 was seen under both reducing (5% 2-mercaptoethanol) (Fig. 4) and nonreducing conditions (not shown). Since the buffer system used for HPLC did not allow monitoring the protein elution pattern by measuring the optical density at 280 nm, we applied proteins of all active fractions

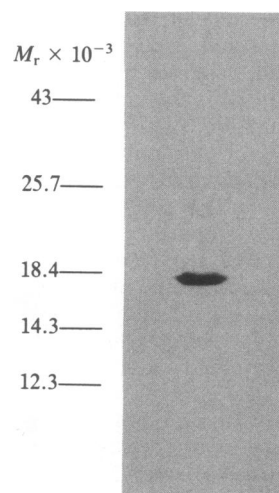


FIG. 4. NaDodSO₄/PAGE. The pluripotent CSF eluted from the HPLC column (200 ng; peak fraction) was lyophilized and treated with 1% NaDodSO₄ in 0.0625 M Tris-HCl, pH 6.8/20% glycerol under reducing conditions (5% 2-mercaptoethanol) for 1 hr at 37°C and then applied to a 15% polyacrylamide gel. After electrophoresis, the protein bands were visualized by the silver staining technique.

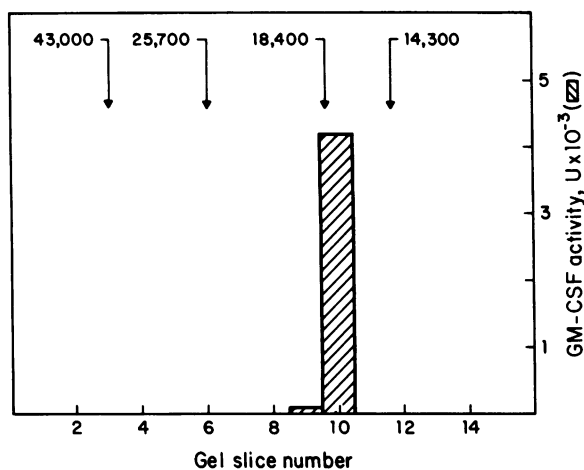


FIG. 5. Preparative NaDodSO₄/PAGE. Pluripotent CSF eluted with HPLC (Fig. 3) was treated and processed (under nonreducing conditions) as shown in Fig. 4. After electrophoresis, the gel was sliced into 4-mm sections and proteins from each slice were eluted into RPMI 1640 medium containing 10% FCS. After 18 hr, eluted proteins were assayed for pluripotent CSF activity (GM-CSF activity: hatched area).

on NaDodSO₄/PAGE. The density of the stained protein band at M_r 18,000 in the peak and side fractions was proportional to the amount of biological pluripotent CSF activity (not shown). After electrophoresis under nonreducing conditions, a parallel gel was sliced into 4-mm sections and proteins were eluted from each slice into RPMI 1640 medium containing 5% FCS. Pluripotent CSF activity was found to be localized in the slice number corresponding to M_r 18,000 (Fig. 5).

In three additional, independent purification runs, pluripotent CSF had the same properties and specific activity as described above. In all three runs parallel gels were sliced into 2-mm sections, and proteins were eluted into P_i/NaCl and tested for pluripotent CSF activity. Re-electrophoresis of the proteins eluted from the slices with pluripotent CSF activity again revealed one single band in a silver-stained gel with a M_r of 18,000, identical to that shown in Fig. 4 (data not shown).

The purified CSF was also subjected to IEF analysis using a 5–60% glycerol gradient in an IEF column and 2% Ampholine (pH 3.5–10). Pluripotent CSF activity was localized in

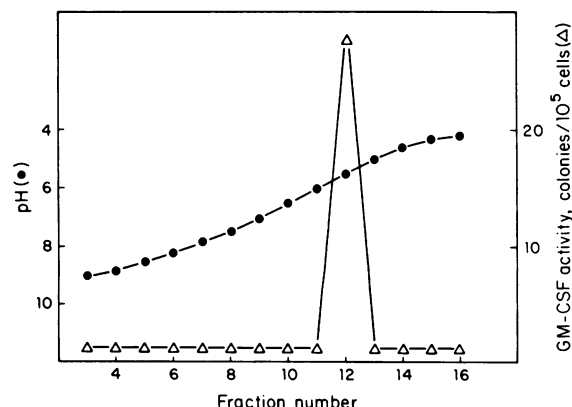


FIG. 6. IEF. HPLC-purified lyophilized pluripotent CSF was supplemented with 20% (vol/vol) glycerol and 2% Ampholine (pH 3.5–10) and layered onto the isodense region of a 0–60% gradient of glycerol containing 2% Ampholine (pH 3.5–10). After IEF (2000 V, 24 hr), 5-ml fractions were collected and the pH (●) was determined in each fraction. All fractions were subsequently dialyzed and tested for pluripotent CSF activity (GM-CSF activity: Δ).

Table 2. Comparison of CFU-GEMM and BFU-E activities of pluripotent CSF (GM-CSF activity, 500 u/ml)

	CFU-GEMM			BFU-E		
	Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 3
Medi-						
um*	0.3 ± 0.3	0	0	42 ± 6	17 ± 3	17 ± 2
LCM†	7 ± 1	3 ± 0	3.3 ± 0.3	67 ± 1	65 ± 3	34 ± 3
PP-						
CSF‡	7.7 ± 2.1	4 ± 0.8	2.3 ± 0.9	85 ± 6	31 ± 1	28 ± 2

Target cells were 5×10^4 per ml of low density, nonadherent, and T-cell-depleted normal human BM cells. Experiment 3 was done in the absence of hemin. The number of colonies is shown as the mean ± SEM.

*Isocove's modified Dulbecco medium plus 30% FCS.

†Medium conditioned by leukocytes from patients with hemochromatosis in the presence of 1% phytohemagglutinin (PHA) (positive control).

‡Pluripotent CSF.

one fraction (5 ml) with an isoelectric point (pI) of 5.5 (Fig. 6). The total recovery of pluripotent CSF activity applied to the column was ≈20%.

Pluripotent CSF activity did not bind to a concanavalin A-agarose. Treatment with neuraminidase did not abolish the biological activity and did not change the pI (data not shown). However, the IEF under our conditions did not allow judgment of minor changes of the pI. These findings suggest that glycosylation might not be a major structural feature.

Biological Activity of Pluripotent CSF. Fifty units of GM-CSF activity, enough to support the half-maximal growth of CFU-GM, had no clear effect in a CFU-GEMM assay; however, 500 u (GM-CSF activity) of pluripotent CSF per ml clearly supported the growth of human mixed colonies (CFU-GEMM) and early erythroid colonies (BFU-E) under our experimental conditions (Table 2). Preliminary results showed that 50 u/ml and 200 u/ml of GM-CSF activity of the pluripotent CSF were needed to induce half-maximal differentiation of the leukemic cell lines WEHI-3B (D+) and HL-60, respectively (data not shown).

DISCUSSION

In this study we describe the purification of a pluripotent CSF, which is constitutively produced by the human bladder carcinoma cell line 5637. This protein is capable of stimulating the *in vitro* growth of mixed colony progenitor cells (CFU-GEMM), early erythroid progenitor cells (BFU-E), and granulocyte-macrophage progenitors (CFU-GM) and, in addition, induces differentiation of both the murine myelomonocytic [WEHI-38B (D+)] and the human promyelocytic (HL-60) leukemic cell lines (unpublished data). The purified pluripotent CSF had a specific activity in the GM-CSF assay of 1.5×10^8 u/mg of protein. To our knowledge this is the highest specific activity for a human pluripotent CSF reported to date. Pluripotent CSF has a M_r of 32,000 by gel filtration and a M_r of 18,000 by NaDodSO₄/PAGE under both reduced and nonreduced conditions and a pI of 5.5. Pluripotent CSF activities could be eluted from gel slices representing the same molecular weight range as the stained protein band.

The purified protein shown in NaDodSO₄/PAGE is consistent with pluripotent CSF because (i) the profile of protein elution visualized in NaDodSO₄/PAGE (not shown) and elution of pluripotent CSF activity (Fig. 3) from RP-HPLC columns is equivalent in the major fraction and side fractions, (ii) additional chromatography of the purified protein on diphenyl or octyl RP-HPLC columns using acetonitrile or ethanol as organic solvents for elution did not lead to a separa-

tion of protein and pluripotent CSF activity (data not shown), (iii) there is identical localization of the protein band and pluripotent CSF activity in a preparative NaDodSO₄/PAGE, and (iv) high specific GM-CSF activity (1.5×10^8 u/mg of protein) occurs. Our data suggest that we have purified pluripotent CSF to apparent homogeneity and, therefore, we have initiated amino acid sequence analysis of our purified protein (data not shown). It is possible, though we believe unlikely, that pluripotent CSF is associated with a minor component representing <5% of the preparation.

Because the M_r of pluripotent CSF is 18,000, it could be calculated that 1 u of pluripotent CSF was equivalent to 6.7 pg of protein or 3.7×10^{-16} mol. A pluripotent CSF concentration of 50 u/ml or 18.5 pM was required for half-maximal colony formation from CFU-GM activity in normal human BM cells.

A 10-fold increase in the amount of pluripotent CSF (GM-CSF activity, 500 u/ml) was required for clear detection of human CFU-GEMM and erythroid BFU-E activities (Table 2); a 1- to 2-fold increase in pluripotent CSF (50–200 u of GM-CSF) was needed to induce the differentiation of either WEHI-3B (D+) or HL-60 leukemic cells, respectively (preliminary data). These data suggest that the particular action(s) of pluripotent CSF are determined by its concentration, as first suggested by Burgess and Metcalf (1) in the murine system. The fact that human pluripotent CSF is able to induce differentiation of leukemic cell lines makes it a protein with unique properties, since for the murine multi-CSF (interleukin 3) no differentiation activity on leukemic cells has been reported (4).

Several human CSFs (GM-CSF, G-CSF, and eosinophilic CSF, erythroid-potentiating activity) have M_r s between 30,000 and 40,000 on gel filtration (7, 9–12), which is similar to the native molecular weight of the pluripotent CSF described here. However, only partially purified erythroid-potentiating activity has been reported to have activity in a CFU-GEMM assay (20).

Constitutive production of pluripotent CSF by the bladder carcinoma cell line 5637 suggests that it is a valuable source for large-scale production and for isolation and cloning of the gene that codes for pluripotent CSF. The availability of purified human pluripotent CSF has important and far-reaching implications in the management of clinical diseases involving hematopoietic derangement or failure.

We thank Dr. J. Fogh for kindly providing us with the 5637 cell line and Mrs. Maureen Sullivan, Mr. John Foster, and Mr. Andrej Povilka for excellent technical assistance. This work was supported by National Cancer Institute Grants CA 20194, CA 23766, CA 32516, CA 33484, CA 33873, CA 34995, and CA 00966 and by Grant CH 251 from the American Cancer Society.

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